

*Listing of Claims*

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1 - 88. (Cancelled)

89. (Currently amended) A method of expanding embryonic stem cells, said method comprising [expanding embryonic stem cells in serum-free culture] contacting embryonic stem cells with a serum-free medium, said medium comprising a basal cell culture medium and a serum-free eukaryotic cell culture medium supplement.

90. (Currently amended) The method of claim 89, wherein said [expanding occurs in the absence of] serum-free medium does not contain leukemia inhibitory factor.

91. (Currently amended) A composition comprising embryonic stem cells in a serum-free eukaryotic cell culture medium, wherein said serum-free medium comprises a basal cell culture medium supplemented with a serum-free eukaryotic cell culture medium supplement, and wherein said medium supports the growth of embryonic stem cells in serum-free culture.

92. (Currently amended) The composition according to claim 91, wherein said [medium comprises a basal cell culture medium supplemented with a serum-free, eukaryotic cell culture medium supplement comprising] serum-free eukaryotic cell culture medium supplement comprises one or more ingredients selected from the group consisting of

albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements.

93. (Currently amended) [The composition according to claim 91] A composition comprising embryonic stem cells in a serum-free eukaryotic cell culture medium, wherein said serum-free medium is obtained by combining a basal cell culture medium with a serum-free, eukaryotic cell culture medium supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements.

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94. (Previously added) The composition according to claim 92 or 93, wherein said composition is capable of being stored indefinitely at less than or equal to about -135°C.

95. (Previously added) The composition according to claim 94, wherein said embryonic stem cells are obtained from an animal selected from the group consisting of human, monkey, ape, mouse, rat, hamster, rabbit, guinea pig, cow, swine, dog, horse, cat, goat, sheep, bird, reptile, fish, and amphibian.

96. (Previously added) The composition according to claim 95, wherein said embryonic stem cells are obtained from an animal selected from the group consisting of mouse, cow, goat, and sheep.

97. (Previously amended) The composition according to claim 96, wherein said embryonic stem cells are murine cells.

98. (Currently amended) A product of manufacture comprising a container means containing embryonic stem cells and a serum-free, eukaryotic cell culture medium supplement, said supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements,

wherein a basal cell culture medium supplemented with said supplement supports the growth of embryonic stem cells in serum-free culture.

99. (Currently amended) A product of manufacture comprising a container means containing embryonic stem cells in a serum-free eukaryotic cell culture medium, said medium comprising a basal cell culture medium supplemented with a serum-free, eukaryotic cell culture medium supplement, said supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more

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antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors,  
and one or more trace elements,

wherein said supplemented medium supports the growth of embryonic stem cells in  
serum-free culture.

100. (Currently amended) A product of manufacture comprising a container  
means containing embryonic stem cells in a serum-free medium obtained by combining a  
basal cell culture medium with a serum-free, eukaryotic cell culture medium supplement,  
said supplement comprising one or more ingredients selected from the group consisting of  
albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or  
more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins  
or insulin substitutes, one or more collagen precursors, and one or more trace elements,

wherein said serum-free medium supports the growth of embryonic stem cells in  
serum-free culture.

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101. (Currently amended) A product of manufacture comprising one or more  
container means, wherein a first container means contains a serum-free, eukaryotic cell  
culture medium supplement, said supplement comprising one or more ingredients selected  
from the group consisting of albumins or albumin substitutes, one or more amino acids, one  
or more vitamins, one or more transferrins or transferrin substitutes, one or more  
antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors,  
and one or more trace elements,

wherein a basal cell culture medium supplemented with said supplement supports the growth of embryonic stem cells in serum-free culture,

wherein [optionally] a second container means contains [a basal medium] embryonic stem cells, and

wherein optionally a third container means contains [embryonic stem cells] a basal medium.

102. (Currently amended) A product of manufacture comprising one or more container means, wherein a first container means contains a serum-free eukaryotic cell culture medium, said medium comprising a basal cell culture medium supplemented with a serum-free, eukaryotic cell culture medium supplement, said supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements,

wherein said supplemented medium supports the growth of embryonic stem cells in serum-free culture, and

wherein a second container means contains embryonic stem cells.

103. (Currently amended) A product of manufacture comprising one or more container means, wherein a first container means contains a serum-free medium obtained by combining a basal cell culture medium with a serum-free, eukaryotic cell culture medium supplement, said supplement comprising one or more ingredients selected from the group

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consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements,

wherein said serum-free medium supports the growth of embryonic stem cells in serum-free culture, and

wherein a second container means contains embryonic stem cells.

104. (Previously added) The product of manufacture according to any one of claims 98-103, wherein said product of manufacture is in a frozen state.

105. (Currently amended) A method of expanding embryonic stem cells in serum-free culture, said method comprising

(a) contacting said embryonic stem cells with a serum-free eukaryotic cell culture medium, said medium comprising a basal cell culture medium supplemented with a serum-free, eukaryotic cell culture medium supplement, said supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements; and

(b) cultivating said embryonic stem cells under serum-free conditions suitable to facilitate the expansion of said embryonic stem cells.

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106. (Currently amended) A method of expanding embryonic stem cells in serum-free culture, said method comprising

(a) contacting said embryonic stem cells with a serum-free eukaryotic cell culture medium obtained by combining a basal cell culture medium with a serum-free, eukaryotic cell culture medium supplement, said supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements; and

(b) cultivating said embryonic stem cells under serum-free conditions suitable to facilitate the expansion of said embryonic stem cells.

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107. (Previously added) The method according to claim 105 or 106, wherein said method further comprises seeding said embryonic stem cells upon a layer of feeder cells.

108. (Currently amended) A method for controlling or preventing the differentiation of embryonic stem cells in serum-free culture, said method comprising

(a) contacting said embryonic stem cells with a serum-free eukaryotic cell culture medium, said medium comprising a basal cell culture medium supplemented with a serum-free, eukaryotic cell culture medium supplement, said supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or

transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements; and

(b) cultivating said embryonic stem cells under serum-free conditions suitable to control or prevent the differentiation of embryonic stem cells and facilitate the expansion of said embryonic stem cells in serum-free culture.

109. (Currently amended) A method for controlling or preventing the differentiation of embryonic stem cells in serum-free culture, said method comprising

(a) contacting said embryonic stem cells with a serum-free eukaryotic cell culture medium obtained by combining a basal cell culture medium with a serum-free, eukaryotic cell culture medium supplement, said supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements; and

(b) cultivating said embryonic stem cells under serum-free conditions suitable to control or prevent the differentiation of embryonic stem cells and facilitate the expansion of said embryonic stem cells in serum-free culture.

110. (Previously added) The method according to claim 108 or 109, wherein said method further comprises seeding said embryonic stem cells upon a layer of feeder cells.



111. (Previously added) The method according to claim 110, wherein said cultivating further comprises supplementing said medium with one or more factors which control or prevent the differentiation of said embryonic stem cells.

112. (Previously added) The method according to claim 111, wherein said factor is selected from the group consisting of leukemia inhibitory factor, steel factor, ciliary neurotrophic factor, and oncostatin M.

113. (Previously added) The method according to claim 112, wherein said factor is leukemia inhibitory factor.

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114. (Previously added) The method according to claim 112, wherein said factor is steel factor.

115. (Previously added) The method according to claim 112, wherein said factor is ciliary neurotrophic factor.

116. (Previously added) The method according to claim 112, wherein said factor is oncostatin M.

117. (Currently amended) A method of causing embryonic stem cells to differentiate into a particular type of cell in serum-free culture, said method comprising

(a) contacting said embryonic stem cells with a serum-free eukaryotic cell culture medium, said medium comprising a basal cell culture medium supplemented with a serum-free, eukaryotic cell culture medium supplement, said supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements;

(b) cultivating said embryonic stem cells under conditions suitable to facilitate the expansion of embryonic stem cells in serum-free culture; and

(c) adding a differentiation factor or changing culturing conditions to induce differentiation of embryonic stem cells to form a different type of cell.

118. (Currently amended) A method of causing embryonic stem cells to differentiate into a particular type of cell in serum-free culture, said method comprising

(a) contacting said embryonic stem cells with a serum-free eukaryotic cell culture medium obtained by combining a basal cell culture medium with a serum-free, eukaryotic cell culture medium supplement, said supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements;

(b) cultivating said embryonic stem cells under conditions suitable to facilitate the expansion of embryonic stem cells in serum-free culture; and

(c) adding a differentiation factor or changing culturing conditions to induce differentiation of embryonic stem cells to form a different type of cell.

119. (Previously added) The method according to claim 117 or 118, wherein said method further comprises seeding said embryonic stem cells upon a layer of feeder cells.

120. (Previously added) The method according to claim 117 or 118, wherein said cultivating said embryonic stem cells under conditions suitable to prevent the differentiation of and facilitate the expansion of said cells further comprises supplementing said culture medium with one or more growth factors which prevent differentiation of said embryonic stem cells.

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121. (Previously added) The method according to claim 117 or 118, wherein said cultivating said embryonic stem cells further comprises supplementing said culture medium with one or more growth factors which facilitate differentiation of said embryonic stem cells.

122. (Currently amended) A method of obtaining embryonic stem cells in serum-free culture, said method comprising

- (a) isolating embryonic stem cells from blastocysts; and
- (b) cultivating said isolated embryonic stem cells in a serum-free eukaryotic cell culture medium, said medium comprising a basal cell culture medium

supplemented with a serum-free, eukaryotic cell culture medium supplement, said supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements,

wherein said supplemented medium supports the growth of embryonic stem cells in serum-free culture.

123. (Currently amended) A method of obtaining embryonic stem cells in serum-free culture, said method comprising

(a) isolating embryonic stem cells from blastocysts; and

(b) cultivating said isolated embryonic stem cells in a serum-free eukaryotic cell culture medium obtained by combining a basal cell culture medium with a serum-free, eukaryotic cell culture medium supplement, said supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements,

wherein said serum-free medium supports the growth of embryonic stem cells in serum-free culture.

124. (Currently amended) A method of producing recombinant protein in embryonic stem cells in serum-free culture, said method comprising

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(a) obtaining a recombinant embryonic stem cell containing a nucleic acid molecule which encodes a protein of interest;

(b) contacting said recombinant embryonic stem cell with a serum-free medium, said serum-free medium comprising a basal cell culture medium supplemented with a serum-free cell culture supplement;

[(b)] (c) culturing said embryonic stem cell in said serum free [culture] medium to form a population of recombinant embryonic stem cells; and

[(c)] (d) isolating said protein from said embryonic stem cells or from the medium in which said cells are cultured.

125. (Currently amended) The method according to claim 124, wherein said isolating comprises

[(c)] d1) isolating said protein from said embryonic stem cells.

126. (Currently amended) The method according to claim 124, wherein said isolating comprises

[(c)] d1) isolating said protein from said [harvested] medium in which said cells are cultured.

127. (Currently amended) The composition of claim 92, wherein said serum-free eukaryotic cell culture medium supplement comprises human albumin or bovine albumin.

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128. (Currently amended) The composition of claim [127] 92, wherein said [human albumin is] serum-free eukaryotic cell culture medium supplement comprises lipid-poor human albumin.

129. (Currently amended) The composition of claim 92, wherein said serum-free eukaryotic cell culture medium supplement comprises human transferrin or bovine transferrin.

130. (Currently amended) The composition of claim [129] 92, wherein said [human transferrin is] serum-free eukaryotic cell culture medium supplement comprises iron-saturated human transferrin.

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131. (Currently amended) The product of manufacture of claim 98, wherein said serum-free eukaryotic cell culture medium supplement comprises human albumin or bovine albumin.

132. (Currently amended) The product of manufacture of claim [131] 98, wherein said [human albumin is] serum-free eukaryotic cell culture medium supplement comprises lipid-poor human albumin.

133. (Currently amended) The product of manufacture of claim 98, wherein said serum-free eukaryotic cell culture medium supplement comprises human transferrin or bovine transferrin.

134. (Currently amended)      The product of manufacture of claim [133] 98, wherein said [human transferrin is] serum-free eukaryotic cell culture medium supplement comprises iron-saturated human transferrin.

135. (Previously added)      The product of manufacture of claim 101, wherein said serum-free eukaryotic cell culture medium supplement comprises human albumin.

136. (Previously added)      The product of manufacture of claim 135, wherein said human albumin is lipid-poor human albumin.

137. (Previously added)      The product of manufacture of claim 101, wherein said serum-free eukaryotic cell culture medium supplement comprises human transferrin.

138. (Previously added)      The product of manufacture of claim 137, wherein said human transferrin is iron-saturated human transferrin.

139. (Previously added)      The method of claim 105, wherein said serum-free eukaryotic cell culture medium supplement comprises human albumin.

140. (Previously added)      The method of claim 139, wherein said human albumin is lipid-poor human albumin.

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141. (Previously added) The method of claim 105, wherein said serum-free eukaryotic cell culture medium supplement comprises human transferrin.
142. (Previously added) The method of claim 141, wherein said human transferrin is iron-saturated human transferrin.
143. (Previously added) The method of claim 108, wherein said serum-free eukaryotic cell culture medium supplement comprises human albumin.
144. (Previously added) The method of claim 143, wherein said human albumin is lipid-poor human albumin.
145. (Previously added) The method of claim 108, wherein said serum-free eukaryotic cell culture medium supplement comprises human transferrin.
146. (Previously added) The method of claim 145, wherein said human transferrin is iron-saturated human transferrin.
147. (Previously added) The method of claim 117, wherein said serum-free eukaryotic cell culture medium supplement comprises human albumin.
148. (Previously added) The method of claim 147, wherein said human albumin is lipid-poor human albumin.

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149. (Previously added) The method of claim 117, wherein said serum-free eukaryotic cell culture medium supplement comprises human transferrin.

150. (Previously added) The method of claim 149, wherein said human transferrin is iron-saturated human transferrin.

151. (Previously added) The method of claim 122, wherein said serum-free eukaryotic cell culture medium supplement comprises human albumin.

152. (Previously added) The method of claim 151, wherein said human albumin is lipid-poor human albumin.

 153. (Previously added) The method of claim 122, wherein said serum-free eukaryotic cell culture medium supplement comprises human transferrin.

154. (Previously added) The method of claim 153, wherein said human transferrin is iron-saturated human transferrin.

155. (New) The method of claim 89, wherein said serum-free medium comprises leukemia inhibiting factor.

156. (New) The composition of claim 95, wherein said embryonic stem cells are obtained from a human.

157. (New) The method of claim 107, wherein said feeder cells are selected from the group consisting of primary embryonic fibroblasts, inactivated feeder cells and STO cells.

158. (New) The composition of claim 92, wherein said serum-free cell culture supplement comprises human albumin.

159. (New) The composition of claim 92, wherein said serum-free cell culture supplement comprises bovine albumin.

160. (New) The composition of claim 92, wherein said serum-free cell culture supplement comprises Albumax®.

161. (New) The composition of claim 92, wherein said serum-free cell culture supplement comprises human transferrin.

162. (New) The composition of claim 92, wherein said serum-free cell culture supplement comprises bovine transferrin.

163. (New) The composition of claim 92, wherein said serum-free cell culture supplement comprises human insulin.

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164. (New) The composition of claim 92, wherein said serum-free cell culture supplement comprises bovine insulin.

165. (New) The composition of claim 92, wherein said serum-free cell culture supplement comprises recombinant insulin.

166. (New) The product of manufacture of claim 98, wherein said serum-free eukaryotic cell culture medium supplement comprises human albumin.

167. (New) The product of manufacture of claim 98, wherein said serum-free eukaryotic cell culture medium supplement comprises bovine albumin.

168. (New) The product of manufacture of claim 98, wherein said serum-free eukaryotic cell culture medium supplement comprises Albumax®.

169. (New) The product of manufacture of claim 98, wherein said serum-free eukaryotic cell culture medium supplement comprises human transferrin.

170. (New) The product of manufacture of claim 98, wherein said serum-free eukaryotic cell culture medium supplement comprises bovine transferrin.

171. (New) The product of manufacture of claim 98, wherein said serum-free eukaryotic cell culture medium supplement comprises human insulin.

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172. (New) The product of manufacture of claim 98, wherein said serum-free eukaryotic cell culture medium supplement comprises bovine insulin.

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173. (New) The product of manufacture of claim 98, wherein said serum-free eukaryotic cell culture medium supplement comprises recombinant insulin.

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